THE JOURNAL OF INVESTIGATIVE DERMATOLOGY
Vol. 44, No. 5

Printed in U.S.A.

Preliminary and Short Report

TUMORS OF THE SKIN: IV. DOUBLE-BLIND STUDY ON EFFECTS OF LOCAL ADMINISTRATION OF ANTI-TUMOR AGENTS IN BASAL CELL CARCINOMA*

EDMUND KLEIN, M.D., HOWARD L. STOLL, JR., M.D., HALINA MILGROM, M.D., ROBERT W. CASE, B.S., HERBERT L. TRAENKLE, M.D., SAXON GRAHAM, PH.D., YONA LAOR, M.D. AND FREDERICK HELM, M.D

During previous observations of the interactions of various cutaneous neoplasms with various antimitotic agents, it was apparent that some of these compounds produced a rapid and sometimes marked alteration in the appearance of basal cell carcinomas on local (1, 2, 3, 4, 5, 6) and parenteral (7) administration. Consequently, a controlled study was initiated to determine the nature and relative degree of the biological changes in basal cell carcinoma resulting from the topical application of several antimitotic agents. Although clinical management of basal cell carcinoma was not the primary concern of the study, one of the parameters of the biologic changes studied was the disappearance of the lesion.

METHODS AND MATERIALS

Initial exploratory studies were carried out to determine possible toxicity and general nature of response to the several anti-tumor agents under consideration. From these agents the group of compounds listed below were selected for a double blind study involving 31 lesions.

The following compounds† were dispensed in Plastibase in the respective concentrations: 0.02% Actinomycin D, 20% Methotrexate, 20% Spiramycin, 0.005% Nitrogen Mustard, 20% 5-Mercaptouracil (AB 050), and 10% Dimethylurethimine (AB 132). 20% 5-Fluorouracil (5-FU) was dispensed in Acid Mantle cream. Unmedicated vehicles (Acid Mantle cream and petrolatum) were used for controls.

The various concentrations were selected on the basis of generally established systemic doses, assuming uniform distribution in various tissues, and on the basis of preliminary observations of the concentrations of the various agents that could be expected to be used without causing severe toxic reactions to the normal skin of the study site. In spite of these precautions in choosing the concentrations of the agents, the applications were discontinued in several subjects because of severe local and/or more widespread reactions.

The protocol established for this study required the following criteria as qualifications for inclusion of a tumor in the study.

1. Nodular basal cell carcinoma without ulceration.
2. Size of tumor no larger than could be easily excised and repaired with primary closure (*i.e.* 1 cm in diameter or less).
3. No previous therapy.
4. Location of tumor at a site that could be easily excised and repaired by primary closure without compromising the patient's appearance or function (excluded nose, naso-labial fold, upper lip, oral commisure, eyelids, and canthi). In accordance with the method outlined in the study protocol, the following procedures were included:

a. Selection of lesion.

b. Photograph of lesion prior to, during, and at termination of study.

c. Punch biopsy specimen prior to and during the study and excision biopsy specimen at termination of study.

d. Administration of agent.

Each agent was tested separately on a random basis and dispensed in sequence. The agent was applied every two days under an occlusive dressing of plastic film for one month. Each application and dressing change was carried out by a member of the clinic. The selection of tumors that satisfied the protocol required exclusion of 90% of the

This work has been assisted by an allocation, IN-54C, from the American Cancer Society Institutional Grant and the Arcade Community Chest.
Received for publication December 4, 1964.

* From the Department of Dermatology, the Department of Pharmacy, and the Department of Epidemiology, Roswell Park Memorial Institute, Buffalo, New York.

† The chemical agents were obtained as follows: 5-Fluorouracil through the cooperation of Dr. E. Miller, Hoffman-La Roche, Inc., Nutley, N.J.; 5-Mercaptouracil and Dimethylurethimine through the cooperation of Dr. T. Bardos, School of Pharmacy, State University of New York at Buffalo and Dr. J. Ambrus, Department of Experimental Biology, Roswell Park Memorial Institute, Buffalo, New York; Spiramycin through the cooperation of Dr. N. Back of the School of Pharmacy, State University of New York at Buffalo; Actinomycin D through the cooperation of Drs. J. Scigliano and A. Osterberg of the Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Maryland; Methotrexate through the cooperation of Dr. J. Rugsegger of the Lederle Laboratories, Pearl River, N. Y.; Nitrogen Mustard as Mustagen, Merck Sharp and Dohme, West Point, Pa.

basal cell carcinomas admitted for treatment to this service.

RESULTS

In 4 basal cell carcinomas Actinomycin D was studied. Three of these tumors decreased very slightly in size during the applications. The fourth tumor underwent superficial ulcerations, but was still grossly evident when the application of Actinomycin D was stopped. One month after discontinuation of Actinomycin D the tumor had been replaced by a smooth intact epithelium. Excision biopsy specimen of the study site showed a patchy lymphocytic infiltrate and new collagen fiber formation without residual basal carcinoma.

In two tumors nitrogen mustard was studied. Both tumors decreased slightly in size and one became superficially eroded, but neither disappeared.

AB 050 was administered to 8 basal cell carcinomas. Six showed eczematization (vesiculation and crusting) of variable severity of the tumor and surrounding normal skin. Administration of AB 050 to 4 of the sites was discontinued because of the appearance of severe dermatitis. Of the 8 tumors studied, 2 were no longer evident grossly or by biopsy examination following administration of AB 050. One of these tumors had been exposed to AB 050 for only two weeks, administration having been stopped because of severe dermatitis. Excision biopsy specimen showed degeneration of collagen fibers, intense lymphocytic infiltration and no tumor. The other tumor that was eliminated was a very small (3 mm) superficial tumor that gradually decreased in size during the application of AB 050. No dermatitis occurred and on cessation of the applications, no tumor was evident grossly or histologically.

In 3 basal cell carcinomas administration of Spiramycin was studied. Applications were discontinued to all study sites in less than one month because dermatitis of tumor and surrounding skin was marked. None of the three tumors was eradicated.

Methotrexate was administered to two basal cell carcinomas. Both tumors decreased slightly in size, one became ulcerated but both were still present grossly and microscopically after cessation of the applications.

In six basal cell carcinomas 5-fluorouracil was studied. In most tumors, erythema became evident within 24–48 hours. Lymphocytic infiltration in small patches around blood vessels became evident within two days. Some tumors increased slightly in size initially; whether this was due to spongiosis and/or edema of the corium could not be determined unequivocally. Following the initial transient swelling, decrease in tumor size became marked and progressive. Continued daily application of 5-fluorouracil under occlusive dressing produced increased erythema but did not cause the severe vesicular dermatitis seen with some of the other agents. Denudation of the epithelium and ulceration through the epithelium appeared in portions of the tumor and usually terminated by involving the whole surface of the tumor. The base of the ulceration was soft, presumably because of necrosis of the tumor that was evident microscopically at that time. Occasionally during the course of 5-fluorouracil therapy, but generally after the applications had been discontinued, a crust formed on the soft ulcerated site. Spontaneous exfoliation of the crust left a firm erythematous depressed surface.

During applications of 5-fluorouracil, erythema usually appeared in the skin surrounding the tumor. Small areas of denudation, a few millimeters in size, sometimes appeared in the erythematous skin surrounding the tumor. Erythema and denudation in the skin around the tumor appeared later and were less marked than in the tumor.

Five of the six tumors studied were no longer evident following administration of 5-fluorouracil. The smooth slightly depressed erythematous sites all showed similar changes on excision biopsy section. Many new small collagen fibers were evident. The increased amount of collagen was consistent with the appearance of early scar formation. Patches of densely packed lymphocytes were scattered within the corium.

One of the tumors studied with 5-fluorouracil decreased in size and depth during the applications so that the study site was flat, but excision biopsy section revealed nests of viable appearing basal cell carcinoma in the corium as well as the other microscopic changes mentioned above.

One basal cell carcinoma was studied with AB 132. Within a few days, severe eczematous dermatitis appeared in the tumor and surrounding area. Application of AB 132 was discontinued and the tumor persisted.

Acid mantle cream was applied to three basal cell carcinomas and petrolatum was applied to two basal cell carcinomas with the same regimen as used for the antimitotic agents. Five tumors were unchanged after applications and showed no alteration microscopically.

DISCUSSION

This preliminary study was undertaken to determine which, if any, of several antimitotic agents would result in measurable biological changes in basal cell carcinoma. By the time observations on 31 tumors had been accumulated, it became apparent that the behavior of the majority of the treated tumors differed markedly from the natural course of basal cell carcinoma.

A reaction to the locally administered agents was present in 26 of 31 tumors, although there were considerable differences in the degree of response. The corresponding 31 excisional biopsy specimens confirmed microscopically the grossly apparent reaction in the 26 lesions (80%) and indicated complete regressions in 25% of tumors. Therefore the code was broken in order to determine whether these effects were associated with

several of the agents under study, or whether statistical significance for a single agent had been established. If the tumors were reacting similarly to several of the compounds, an extension of the study would be necessary; whereas, if the tumors were reacting more markedly to one compound than to the others, the study could be revised for a more intensive investigation of the active compound. Those tumors on which the investigation was completed in accordance with the protocol were evaluated by Chi square analysis to determine whether one of the agents caused a significantly greater number of tumor regressions than the other agents. The difference between the number of tumor regressions after application of 5-fluorouracil and after application of the controls was significant at the 5% level (P less than .001). The difference between the number of tumor regressions after application of AB 050 and after application of the controls was not significant at the 5% level. The controls used for these analyses were the unmedicated bases and the medicated preparations other than 5-fluorouracil and AB050.

The study was designed to conform with conditions previously found to be suitable for further clinical studies. The statistical significance established for the action of 5-fluorouracil is primarily relevant for the selected parameters of this protocoled study. The lack of established statistical significance for the activities of the other agents in this study does not by itself exclude anti-tumor action against basal cell carcinoma on the part of these compounds. In fact, each one of the agents which appeared to be less active than 5-fluorouracil had anti-tumor activity against basal cell carcinoma when the conditions were modified. Thus, topical administration of methotrexate to multiple superficial basal cell carcinomas was followed by complete regression of the lesions following an inflammatory response sharply limited to the site of tumor involvement. Similarly, actinomycin D, nitrogen mustard, AB 050 and AB 132 were shown to have anti-tumor activity against basal cell carcinoma when the conditions of the study were altered.

In accordance with the objectives of the protocol for this study, factors such as concentration, penetration, absorption, vascular supply, and rate of removal, which are of critical pharmacologic importance, have not been specifically considered in this study.

Definitive or long-range evaluation of the various agents was limited by the duration of the observation period set by the protocol criteria and rendered nonfeasible on a long term basis by the need for excisional biopsy studies as the terminating step of the procedure. On the basis of this data, however, a more definitive study of 5-fluorouracil was initiated which included evaluation for protracted periods of observation.

SUMMARY AND CONCLUSIONS

A double blind controlled study on the reaction of basal cell carcinoma to the local administration of several antimitotic agents was carried out. Under these experimental conditions, 5-fluorouracil had significant therapeutic effect as determined by the incidence of tumor regressions on excisional biopsy examination.

REFERENCES

1. Belisario, J.: Recent assessments in the aetiology and therapy of skin carcinomas. Indian J. Derm. Vener., *29:* 191, 1963.
2. Goldman, L.: The response of skin cancer to topical therapy with 5-fluorouracil. Cancer Chemother. Rep., *28:* 49, 1963.
3. Dillaha, C. J., Jansen, G. T., Honeycutt, W. M. and Bradford, A. C.: Selective cytotoxic effect of topical 5-fluorouracil. Arch. Derm. (Chicago), *88:* 247, 1963.
4. Klein, E., Milgrom, H., Helm, F., Ambrus, J., Traenkle, H. L. and Stoll, H. L.: Tumors of the skin: effects of local use of cytostatic agents. Skin, *3:* 81, 1962.
5. Klein, E., Helm, F., Milgrom, H., Stoll, H. L. and Traenkle, H. L.: Tumors of the skin—II: Keratoacanthoma; local effects of 5-fluorouracil. Skin, *1:* 153, 1962.
6. Stoll, H. L., Milgrom, H. and Klein, E.: Tumors of the skin—III: Lymphoma of the skin; local effect of triamcinolone. Skin, *1:* 225, 1962.
7. Van Scott, E. J., Shaw, R. K., Crounse, R. C. and Condit, P. T.: Effects of methotrexate on basal cell carcinomas. Arch. Derm. (Chicago), *82:* 762, 1960.